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PHARMACOLOGIC MANIPULATION OF DERMAL ISCHEMIA IN THERMAL INJURY

Henry George Stebbins Noble
B.A., Yale University, 1971

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TABLE OF CONTENTS

Page
Abstract 1
Introduction 2
Anatomy of Skin and its Microcirculation 5
Skin as Altered by Thermal Injury 9
Materials and Methods 19
Results 23
Discussion 26
Summary 39
Figures 40
Appendix 52
References 53

ABSTRACT

The circulation of the skin is markedly altered by thermal trauma. Order and his associates studied burned skin in rats by angiographic techniques similar to those described in these experiments. They emphasized that burns caused ischemia almost immediately by arteriolar occlusion. Jackson has suggested that vascular changes are responsible for the conversion of the zone of stasis during the first 24 hours post burn.

In the present series of experiments, the concept that the changes seen in the zone of stasis could be prevented or reversed by pharmacologic manipulation was examined. Using antisludging agents, anticoagulants, and vasoactive agents the thermally injured skin vasculature was examined by angiography.

Arteriography was performed via the thoracic aorta in Sprague-Dawley rats immediately, and at 1, 2, 4, 6, 24, 48 and 72 hours following a 20% full thickness scald burn. In control burns, the major arterial occlusion occurred between 2 and 4 hours post burn and the wound was totally avascular by 24 hours. This was prevented by continuous heparinization if started prior to burning and was significantly improved by phentolamine. Low molecular weight dextran appeared to worsen the arteriographic appearance of the wound, whereas chlorpromazine and dilute epinephrine did not significantly affect it.

These results suggest that the "zone of stasis" may be able to be pharmacologically manipulated if the proper combinations of agents can be given at the proper time to stop or reverse the vascular changes now seen in the burn wound.

INTRODUCTION

Burns are a common form of trauma which is not restricted to any particular segment of the population. The injury may be thermal, radiant, electrical or chemical. Of these, the most common are thermal. Among non-transport accidents, burns rank as the leading cause of death in the 1-4 year old age group and the over 45 year old age group. In the group from age 5 to age 14, burns are the second leading cause of death in non-transport accidents. Lewis[34] estimates that there are about 2,000,000 burn injuries per year in this country. Of these, about 70,000 are hospitalized for their burns. Approximately 12,000 people die of their burns. This estimate includes those who die before reaching the hospital. Among those who reach the hospital alive, death may still occur many days post injury despite intensive therapy.

While mortality is significant, morbidity among those who survive or who suffer lesser injuries is even more important. The unique features of the burn wound related to the extensive gap in the integument which cannot be closed simply, lead to prolonged exposure to the dangers of infection and systemic physiologic derangements which may be life threatening. These features also contribute to the protracted painful recovery. Furthermore, the end result may be physically deforming, leaving the patient with psycho-social scars as well. The cost in human suffering of the burn is very high.

The study of burn injury has become a model for the study of trauma. Yet in many ways intensive investigation of the burn has lagged behind other medical research.

> The burn wound, although representing a damaged organ, has not received the intense morphologic investigation that has been demanded in other disease states. The exterior mutilation caused by the injury has discouraged interest and impeded proper examination. Perhaps nowhere in modern medicine has visual displeasure so hindered objectivity and analysis.
>
> (Preface from The Burn Wound[43])

Major events in the therapy of burns have been the appreciation of the importance of fluid loss in 1921, the development of grafting as we know it today in 1930, the development of fluid and electrolyte therapy following the Cocoanut Grove fire in 1942, and the development of antibiotics to deal with burn sepsis beginning with penicillin in 1945. All of these therapies are designed to correct the body's responses to the injury and its systemic effects. Fluid and electrolyte therapies are mainly supportive, replacing that which is lost through the open integument, until the patient's hemodynamics are restored to normal. Antibiotic therapy has been effective in preventing further external injury to the burn wound. Order and Moncrief[43] demonstrated that bacterial infection of the burn wound will transform a second degree burn to a full thickness wound. However, if, as is assumed in this work, the pathologic processes of the burn continue for some time after the episode of thermal exposure, little if any therapy is aimed specifically at altering these on-going processes.

In this work, it is assumed that somewhere in between tissues which are unaffected by the burn and tissues which are irreversibly damaged, lies tissue which is injured such that, like the patient himself, it may survive the insult or go on to die depending on the success of the therapy at restoring normal conditions. It is further assumed that much of this

tissue is destined to die because available therapies do not control all sources of on-going injury. In this work, an attempt is made to alter the microcirculation of the burn so as to support these marginally viable tissues much as the severely burned patient himself is supported with massive infusions of fluid and electrolytes. This work represents only one approach to hastening the restoration of normal tissue physiology.

ANATOMY OF SKIN AND ITS MICROCIRCULATION

The skin is one of the largest and most important organs of the body. It is the interface between our cells and our environment, acting both to protect us from and to keep us in touch with the environment. It is perhaps the only major organ which must be kept intact if we are to survive. We can survive without a gland or a kidney. We can remove portions of lung, G.I. tract, or brain without endangering survival. But fluid and antibiotic therapy will not be sufficient to ensure the survival of a human deprived of substantial portions of his integument. Ultimately, the opening must be closed in order for him to survive.

The skin exhibits striking variability to correspond with the many functional demands made upon it. It is smooth in some parts, rough in others. It is fixed and taut over the limbs yet loose and elastic over the joints. Some parts are hairy. Others are not. Pigment, vascularity, and thickness vary with the need. Despite the many differences, the structure is basically the same.

The skin is a layered structure. Most superficial is the epidermis. This layer may vary in thickness depending on the need. The added thickness consists of increased superficial layers of keratinized cells. At the base of this layer is a layer of living, regenerating cells from which cells proceed to the surface losing their nuclei and becoming progressively more keratinized over the course of their trip. Eventually, they reach the surface as dead tissue where they are sloughed in the daily wear and tear of the human integument. This is the layer which must be regenerated or replaced if lost in order for a person to survive. It does not arise de novo. It must come from other living epidermal tissue. Healing,

therefore, takes place only at the margins of the wound or progressively outward from islands of remaining epidermal tissue if present.

The junction between epidermis and dermis is not a simple plane but more or less convoluted, depending on function. It is perforated by the various appendages of skin, the hair follicles and sweat glands. These carry epidermal elements with them to a level well below that of the rest of the basal epithelial stratum.

The dermis is a relatively hypocellular layer composed primarily of connective tissue. It acts as a physical support for the epithelium and the glands, nerves, hair follicles, and blood vessels which pass through it. The relative amounts of elastin and collagen and the type and amount of ground substance determine the characteristics of this support. With the subcutaneous fat, the dermis also acts as a cushion for the epidermis. The ground substance in which the collagen is laid may act as a fluid buffer expanding and contracting with changes in superfluous water, electrolytes, sugars, proteins and metabolic products of parenchymal and connective tissue cells. As such, it acts as a gelatinous extension of the circulatory system. Set in this gel are the cells of the dermis, the macrophages, fibroblasts, mast cells and stray white cells. Their function is primarily to maintain the connective tissue barrier and to cope with whatever successfully penetrates the epithelial barrier. The dermis is laid atop domes of subcutaneous fat and projects down between them. Many of the glands and hair follicles pass through the dermis into the tops of these fat domes.

The vascular supply is greatly in excess of what one might expect for this relatively hypocellular tissue. Most investigators agree that the function of the excessive cutaneous vasculature is primarily temperature

regulation. It probably also acts as a buffer reservoir for the systemic circulation. The cutaneous circulation takes the form of a complex meshwork of interconnecting large and small vessels supplied by musculocutaneous perforating arteries but organized into no discrete plexuses. The epidermis takes its nutrition from subpapillary capillary loops. It has no circulation of its own.

Zweifach[57] envisions the organization of the cutaneous vasculature as having a preferential thoroughfare channel connecting arteriole to venule. Extending off this metarteriole are the precapillary sphincters leading to the capillary bed served by this vascular unit. In his view, as sympathetic stimulation increases, the precapillary sphincters contract finally closing off the capillary bed from the circulation. With increasing stimulation, the metarteriole contracts down to stop flow through the preferential channel. Back pressure then forces open an arteriovenous anastomosis. This view, in particular the existence or action of the precapillary sphincters, is not universally accepted.

Arteriovenous anastomoses (AVA's) are probably present in the microcirculation throughout the body. Sherman[53] demonstrated their presence in virtually every part of the skin, not just in acral portions as originally supposed. AVA's may be direct juxtapositions of muscular arteriolar and venular segments or they may be longer connections with a connective-tissue-like tube between recognizable arteriolar and venular segments. Within the AVA's may be found vascular epithelioid cells. These cells are felt to be related to the cells of the carotid and aortic bodies and possibly also the cells of the juxtaglomerular apparatus in the kidney. High levels of acetylcholine can be demonstrated in these cells.

The AVA is surrounded by a rich plexus of nerves, mostly nonmyelinated fibers from the autonomic arterial plexus. Many of these nerves may be cholinergic although construction of the AVA seems to be caused by adrenergic stimulation. Hypoxia will also cause constriction of affected AVA's. AVA's may be dilated by histamine, trauma, increased pressure both venous and arterial, and, in the wall of the stomach, vagal stimulation.

Reinisch[47] has shown, in contrast to Zweifach's view, that AVA's in a sympathectomized skin flap may be selectively constricted by local injection of very dilute epinephrine. He has evidence to show that this stimulation will close the AVA forcing the circulation to pass into capillary beds through precapillary sphincters which, if they exist, must not have been as sensitive to the sympathetic stimulation. This capillary perfusion leads to increased tissue survival.

In the oversized skin flap, some tissue at the distal end must die. In the tissue destined to die, Reinisch is able to demonstrate the presence of vascular flow yet the tissue eventually dies. He postulates that meaningful exchange of oxygen and waste can only occur across capillary walls. The flow demonstrated distal to the surviving tissue is assumed to be only arteriolar and venular connected by patent AVA's, bypassing the capillary circulation.

SKIN AS ALTERED BY THERMAL INJURY

In the pathologic state represented by the burn wound, various amounts of tissue are irreversibly damaged depending on the extent and intensity of the thermal stimulus.[23,40] Other tissue may be seriously damaged but not immediately destroyed and still other tissue may be only transiently affected. The depth of the burn is the critical element in deciding whether a burn wound may heal spontaneously (reepithelialize) or require grafted skin for closure. As we have seen, the epidermis must ultimately be intact for the animal to survive. Most of the regenerating epithelial cells are very near the surface of the skin protected only by the layers of cornified cells superficial to them. Fortunately for the burned patient, epithelial elements extend much deeper into the dermis and beyond to the top of the layer of fat (Figure 1). Epithelial regeneration takes place from these as well as from the margins of the wound. Obviously, the deeper the burn, the fewer the remaining sources of regenerating epithelium until a level is reached, near the bottom of the dermal-fat junction where there are no longer any sources of regenerating epithelium.

Jackson[27,28,29,30] has been concerned with the diagnosis of the depth of burning as an aid to planning therapy and predicting outcome. In 1953, he gave the now classic description of the burn wound as seen from the surface. While not a good method for diagnosing the depth of the burn, it gives a good framework for understanding the pathophysiology of the burn wound. He describes three concentric zones of a significant thermal injury related to the intensity of the thermal stimulus (Figure 2). He points out that this description is based on surface appearances only but states that he believes this appearance to be reflection of the condition of the

superficial dermal capillaries and the blood in them.

The outermost zone, the zone of hyperemia, is the area which has suffered the least injury. From the surface, this zone appears redder than normal skin, blanches with pressure, and becomes cyanotic if a tourniquet is applied proximal to it. In this zone, healing is complete usually by the seventh day. These findings are taken to mean that the dermal circulation is intact with normal to slightly increased flow. Biopsy shows that the superficial epidermis may be lost but the subpapillary circulation is undamaged and patent.

The intermediate zone (of stasis) appears red and blanches on pressure like the outer zone. Application of an exsanguinating tourniquet causes the tissue to blanch and removal leads to return of the redness. This is taken to mean that the subpapillary plexus is in communication with the general circulation. However, application of a tourniquet proximal to the wound does not lead to cyanosis suggesting that oxyhemoglobin present is not being reduced in these tissues. After 24 hours, the tissue fails to blanch with pressure and the exsanguinating tourniquet indicates that this zone is now isolated from the general circulation. Over the next seven days, this tissue becomes indistinguishable grossly from the central zone of coagulation. At this point, on biopsy, the dermal vessels are shown to be choked with red cells.

The central zone of the burn is white and coagulated from the beginning. Biopsy shows obliteration of vascular lumena. Jackson postulates that coagulation of the tissue occurred before the vessels recovered from an initial blanching response to the stimulus. This tissue is presumed to be irreversibly damaged.

Because the zone of stasis changes to become like the zone of coagulation, full thickness skin loss is predicted in any injury where the zone of stasis projects below a line through the junction between dermis and subcutaneous fat, even if the central zone of coagulation does not extend this far.

Many investigators have shown that following the burn injury itself, changes continue to occur in the affected tissue for some time. Whether these changes are manifestations of or contribute to on-going damage, or whether they are evidence of reparative processes is not always clear. However, most investigators agree that significant tissue necrosis occurs for some time after the initial insult. Jackson touched on these changes in his description of the burn wound with the zone of stasis changing over 24-48 hours to become devitalized like the central zone of coagulation. Order and Moncrief[43] touched the subject when they demonstrated arteriographically that the wound was devascularized by 24 hours post injury although an earlier picture had shown patent vessels. Hinshaw[23] describes progressive destruction of epithelial elements in follicles and sweat ducts over 24-48 hours. Some destruction was evident at his first biopsies at 4 hours. Cotran and Remensnyder[15] describe three types of small vessel reaction to thermal injury depending on the intensity. The first, "immediate-transient" response, affecting mildly injured tissue, is over within 30 minutes post injury. The "delayed-prolonged" response to more intense injury begins 15-75 minutes post injury, peaks at about 4 hours, and subsides by 8 hours after the burn. The most intensely burned tissue undergoes an "immediate-sustained" response affecting all vessels until tissue necrosis is more or less complete at 24 hours. Ham and Hurley[22] observed progressive endothelial injury over 24 hours developing by 3-4 hours. Other changes are

observed for 7 days, many presumed associated with repair. Branemark, Breine, Joshi and Urbaschek[8] noted a decrement in flow through the small vessels within 5-7 minutes of the injury. Massiha and Monafo[37] demonstrated immediate compromise of the venous side of the microcirculation which was progressive for 24 hours. It is not clear whether all or some of these changes represent simple manifestation of damage already done or on-going mechanisms of destruction post injury. It does seem clear that the burn wound changes progressively for the time of injury for at least 24 hours.

Many investigators have studied vascular changes in the burn wound assuming that tissue deprived of its circulation must die. Order and Moncrief[43] demonstrated complete devitalization of third degree burn wounds by 24-48 hours post burn. Studying the arterial side of the microcirculation, they demonstrated that this devitalization was progressive, being less severe shortly after the burn, and that some arterial compromise was at least transiently apparent in less severely burned tissue.

Massiha and Monafo[37] studied both the arterial and venous sides of the circulation by similar technique. Their results indicate that the venous side of the circulation reacts immediately and more severely to the thermal injury than the arterial side. They feel that this evidence fits well with Jackson's concept of the zone of stasis and with Order's observations. However, they postulate that arteriolar occlusion is secondary to capillary and venous stasis. Venography consistently showed more extensive damage than arteriography in similar injuries. Beading and tortuosity were evident venographically in even the most superficial burns. Damage was progressive up to 24 hours. Venographic abnormalities were most severe during the first 96 hours post burn. Arteriography showed little or no abnormality in less severe burns. In more severe burns, arteriographic

abnormality was less dramatic than venographic changes up to the degree of injury where the wound showed no patent vessels.

Hurley, Ham and Ryan[26] studied burn vasculature by injection of carbon particles intravenously. These do not pass through the basement membrane and should not cross a healthy endothelium either. Vessels become labelled when there are endothelial gaps allowing leakage of material from the vascular lumen. In the first 30 minutes post burn, Hurley, et al, demonstrated labelling of venules throughout the area. From 30-60 minutes post burn, the pattern changed such that labelling was much diminished but more obvious over the burn site. At this time, capillaries first demonstrate labelling. Labelling increases up to 4 hours post burn. During this time, it is confined to the area of the burn and more intense in the capillaries than in the venules.

Cotran and Remensnyder[15] also studied vascular permeability following thermal injury. They document three types of response. The immediate-transient response, occurring in the first 30 minutes in mild injury, involves only the medium sized venules. This response is said to be identical to that caused by histamine or 5-hydroxytryptamine release in the area. It can be blocked by the administration of an antihistamine. Next, there is a delayed-prolonged response beginning 15-75 minutes post injury which is usually gone by 8 hours. There is widespread involvement of capillaries as well as venules. This response is not responsive to antihistamine. Electron micrographs demonstrate endothelial discontinuities with wide separation of endothelium and the basement membrane. In addition, some endothelial cells appear swollen with cytoplasmic changes suggestive of chemical as well as heat injury. After severe injury, the immediate-sustained response involves exudation from all types of small vessels

including the arterioles. Between 3 and 5 hours, venules and capillaries are demonstrated to be static.

Brooks, Dragstedt, Warnet and Knisley[10] observed the behavior of the microcirculation in vivo following a burn. They listed seven degrees of vascular response: 1) complete health with intact endothelium and smooth laminar flow; 2) mild injury -- temporary sticking of granulocytes to the endothelium; 3) prolonged sticking of the granulocytes with up to two layers coating the endothelium but without emigration of cells from the vessel; 4) prolonged sticking with cells emigrating into the tissue across the endothelium -- small veins dilated in sausage-shaped segments; 5) localized extravasation of erythrocytes and partial collapse of previously bulging endothelium; 6) hemorrhage through the endothelium; and 7) complete disintegration of the endothelium. These authors felt that stages 1-5 could be reversible but 6-7 represented irreversible damage. Brooks, et al, also observed agglutination of red cells as part of the pathologic process. These agglutinations were felt to be larger and more rigid than rouleaux. One population of these could not be broken up to fit into small vessels. Another population was larger, being made up of aggregates of the former and could be broken down to the original agglutinations. These agglutinated red cells were capable of plugging terminal arterioles, forming microthrombi and causing tissue anoxia.

Branemark, et al,[8] also commented on the intravascular events following a burn. They noticed the early dilation and stasis of venules commented upon by earlier mentioned investigators. They also noted arteriolar constriction and the swelling of endothelial cells in the capillaries. By Pouseille's Law, flow varies as the fourth power of the radius of the lumen. Decreasing the arteriolar and capillary lumen in this way dramatically

reduces the nutritive flow. Dilation of the venules would lead to a significant decrease in the velocity of flow in these vessels to the point where stasis is able to occur because, in this non-Newtonian fluid, viscosity increases as velocity decreases. Intravascular contributions to this process are the sticking of red cells, granulocytes, and platelets to the endothelium, loss of plasticity of red cells and granulocytes increasing viscosity of blocking capillaries, and formation of microthrombi of platelets and fibrin, sometimes enmeshing red cells. In addition, Branemark, et al, saw mast cells disrupting, liberating their contents into tissue adjacent to the vessels. As a result of these changes, they recorded a reduction of corpuscular flow velocities in arterioles and venules within 5-7 minutes post burn.

Robb[48] observed dilation of arterioles as well as venules in first degree change with increasing thermal injury. The second degree change was characterized by deposition of platelets on venule walls. This was followed by platelet aggregation, thrombosis, and stasis. Arteriolar circulation continued pulsatile after stasis occurred in the venules. More severe injury resulted in thrombosis and stasis of arterioles as well as venules. The most severe injury resulted in complete destruction of the microcirculation.

Other mechanisms thought to be involved in the pathophysiology of burns include abnormal clotting, toxins and dehydration. Guest and Bond[21] found the microcirculation of the mesentery altered in the exact same way following a severe scald of the skin as it was following an infusion of thromboplastin. This evidence indicates that the burn may touch off the clotting mechanism in some way leading to the clotting abnormalities observed clinically[17] and contributing to the stasis which precedes tissue

anoxia and necrosis. Algower, Cueni, Stadtler and Schoenenberger[1] isolated a "toxin" from burned tissue which was lethal when injected in healthy animals. The mechanism of action was not elaborated. Finally, Zawacki[56] believes that dehydration is a major element in the pathophysiology of burns. He achieved "reversal" of the zone of stasis in burned animals by covering the wound with split thickness porcine graft, while full thickness necrosis was observed in animals treated with removal of the blister covering. Intermediate results were obtained with blisters left intact.

The concept of "reversal" is the big question here. To what extent may Jackson's zone of stasis be reverted to healthy tissue with intact circulation, and by what means? Or, is tissue necrosis inevitable from the beginning? Jackson[27] noticed that the tissue of the zone of stasis, unlike healthy tissues, does not become cyanotic when a tourniquet cuts off its blood supply. The failure to reduce oxyhemoglobin may indicate irreversible biochemical damage with necrosis only a matter of time. On the other hand, as Reinisch showed, this altered metabolism could be due merely to failure of oxygenated blood to pass into the nutritive capillaries. Jackson went on to show that zone of stasis tissue showed much diminished respiration, diminished collagen and ground substance biosynthesis, and death of epithelial elements. Hinshaw[23] discusses the concept of "latent injury," a period of about six hours post burn when the tissue is uniquely susceptible to further injury. It is not clear to what extent any of the previously discussed mechanisms of thermal injury contribute to this "latent injury" or whether the end of this six hour period is equivalent to a reversal of some of the pathologic processes. Perhaps the zone of stasis converts to necrotic tissue because of additional tissue trauma which we have not detected. Brauer and Spira[9] demonstrated that there is

salvageable tissue in the burn by transferring burned skin to a newly denuded bed where the tissue survived. Lest one conclude that necrosis is secondary to compromise of the dermal vascular bed, it should be pointed out that Brauer and Spira also transferred unburned skin grafts to the burn site with good tissue survival. Jackson marvels that zone of stasis tissue, even where full thickness and destined to necrose and slough if untreated, is capable of supporting an isograft with an unexplained reappearance of circulation after four days.[28] Sometime later, the graft sloughed, presumably because of unsuppressed regenerating epithelium beneath it.

These observations are, at our present level of understanding of the burn wound, unexplainable. They do, however, give a clear indication that there is redeemable tissue in the burn wound. In this work, an attempt was made to reproduce the findings of Order and Moncrief,[43] with particular attention to early changes in the arteriographic appearance of the burn wound, and then to modify the changes observed by administration of various pharmacologic agents. A standard 20% full thickness burn was used. Arteriography was performed using a technique similar to that of Order and Moncrief. Animals were studied at 1, 2, 4 and 6 hours post burn as well as the immediate and 24 hour post burn groups studied by Order and Moncrief. Pharmacologic agents were chosen for their known pharmacologic properties which were felt to be potentially effective at altering the pathyphysiology of the burn as presently understood. Heparin was tried for its anticoagulant, antihistaminic, and possible electrostatic effects. Phentolamine was chosen for its notable vasodilating effect. Chlorpromazine was chosen because of its alpha blocking effect and because Horn and Converse[24] had demonstrated dramatic early survival in lethal burns using this agent. Dextran was chosen for plasma expanding properties, electrostatic viscosity-

reducing properties, and a possibly separate antithrombogenic activity. Finally, epinephrine was tried in the hope of reproducing, in burns, the success Reinisch[47] achieved with oversize skin flaps preserving distal tissue perfusion. It was hoped that one or more of these agents would be effective at preventing the progressive loss of vascular filling seen in the burn wound by Order and Moncrief. This group of agents does not by any means exhaust the supply of agents which might be tried, much less the combinations which might prove effective where the individual agents failed when used alone.

MATERIALS AND METHODS

The Burn

Male Sprague-Dawley rats, weighing 200-250 grams, were anesthetized with pentobarbital injected intraperitoneally and the backs were shaved. The anesthetized rat was given a standardized burn by placing him on a template suspended over actively boiling water (100°C) for 15 seconds. This technique has been used in this lab to produce full thickness burns approximating 20% of the animal's body surface. Animals were sacrificed at intervals of 0, 6, 24, 48 and 72 hours post burn with an overdose of pentobarbital.

Injection

Post mortem, the animals were injected with a micropaque-mucilage acacial-gelatin mixture (See Appendix). This is a radiopaque dye which is liquid at 37°C but solid at 20°C. The dye was kept at 37°C in an incubator while the animal was prepared. A midline incision was made and the aorta exposed. The descending aorta just distal to the arch was cannulated with PE 160 tubing filled with normal saline to prevent introduction of bubbles (Figure 3A). Then 6-8 cc. of the micropaque mixture was injected slowly by hand from a syringe. The inferior vena cava was transected above the diaphragm to reduce back pressure from the venous side. The injected animals were cooled for 20-30 minutes at -5°C. Once the gel had been allowed to set, the burned skin was excised with a margin of unburned tissue at a depth just below the panniculus carnosus (Figure 3B). The specimens were preserved in 10% formaldehyde for 2-72 hours with no variation in the quality of the angiograms obtained from them.

Roentgenography

Specimens were laid flat, fur-side down, on Kodak RP-M mammography film in a paper cassette. Exposures were taken at 20 KV, 500 MAS with a focus-film distance of 36 inches using a Beryllium window unit. The roentgenograms were developed in the standard X-O-Mat machine.

Experimental Groups

1. Burn Control. Forty rats were burned in the standard manner and returned to their cages with food and water ad lib. They received no therapy for their burns. Five animals were sacrificed and injected with micropaque at each time interval. Animals were sacrificed immediately and at 1, 2, 4, 6, 24, 48 and 72 hours post burn.

2. Heparin-I. A group of 20 animals were given 500 units of heparin (.5 cc Eli Lilly 1,000 units/cc) subcutaneously immediately post burn. They were then returned to their cages with no further therapy until sacrificed. Groups of five animals were sacrificed immediately and at 6, 24 and 48 hours post burn.

3. Heparin-II. A group of 25 animals were pretreated with three injections, eight hours apart. They were given 200 units (0.2 cc of 1,000 units/cc) subcutaneously each time. They were burned approximately six hours after the third injection. At that time they were demonstrated to have a clotting time in excess of 8 minutes compared to 2 minutes for an untreated control. Heparin was continued in the same dose on the same eight hour schedule post burn until the time of sacrifice. At sacrifice time, animals continued to demonstrate clotting times in excess of eight minutes. Five animals were sacrificed immediately and at 6, 24, 48 and 72 hours post burn.

4. Heparin-III. A group of 15 animals were maintained on the same dose (200 units subcutaneously) on the same schedule (every eight hours) post burn as the rats in the heparin-II group. They were not, however, pretreated for 24 hours. The first dose was given immediately after the burn (less than two minutes after). Subsequent doses followed at eight hour intervals until sacrifice. Animals were sacrificed at 6, 24 and 48 hours post burn. At that time they had clotting times in excess of four times the control value.

5. Phentolamine. A group of 20 rats were given phentolamine in a dose of 2.5 mg/kg intramuscularly in the thigh immediately after the burn and every six hours thereafter until sacrificed. This dose was used by Palmer[44] in his studies of skin flap survival. No other therapy was given. No effort was made to monitor or maintain blood pressure. Groups of five animals were sacrificed immediately, and at 6, 24 and 48 hours post burn.

6. Dextran-40. A group of 15 rats were anesthetized with pentobarbital and the left femoral vein was cannulated with polyethylene tubing. While still anesthetized, the rat was burned with the cannula in place. Immediately following the burn, the animal received 0.55 cc dextran 40 via the cannula. Each animal received an additional 2.5 cc over the next 20-30 minutes. This dose was chosen to give a large dose of dextran without causing cardiac failure due to fluid overload. At the end of the injection the cannula was removed, the vein tied off, and the groin incision closed. No further therapy was given. Groups of five animals were sacrificed and injected with micropaque at 6, 24 and 48 hours post burn.

7. Chlorpromazine. A group of 20 rats received 1.5 mg/kg chlorpromazine intraperitoneally immediately post burn and at 12 hours post burn.

This dose was shown by Horn and Converse[24] to be effective at preventing death in the first 24 hours after lethal burns. At 24 and 36 hours post burn (where applicable) the dose was halved to 0.7 mg/kg. No other therapy was given. Groups of five animals were sacrificed and injected with micropaque immediately and at 6, 24 and 48 hours post burn.

8. Epinephrine. A group of 20 rats were anesthetized. Using an operating microscope, a polyethylene cannula was introduced into the left external jugular vein and tied in place. The protruding end was burrowed under the skin and brought out through a stab wound just caudal to the occiput at the midline where it was plugged and sutured in place. Still under anesthesia, the rats were burned in the standard manner. Immediately post burn, the rats received an injection of 2 cc of a 1:4,000,000 dilution of epinephrine in saline prepared previously. This dilution was used effectively by Reinisch[47] in his study of skin flap circulation. For six hours after the initial injection, each animal received 1 cc hourly of this solution. They received one additional dose of 1 cc at 24 hours post burn. Groups of five rats were sacrificed and injected with micropaque immediately and at 6, 24 and 48 hours post burn.

RESULTS

Unburned Control

In the unburned rat, the skin of the back is supplied by paired perforating musculocutaneous arteries. Order and Moncrief[43] say there are 4; Massiha and Monafo[37] report up to 10. The specimens in this study had 4 or 6 depending on the length of the specimen (Figure 4). These arteries branch multiple times to form a plexus of a uniform density. The arteries of both sides are in communication across the back via this plexus. Although micropaque is said to pass through patent capillary beds to enter the venous circulation, dye was not visualized in the venous circulation in these specimens. There is regular tapering of these arteries as they progress to their distal branches.

Experimental Groups

1. Burn Control. Immediately following a burn, in untreated animals, the arteriographic picture cannot be distinguished from that of an unburned animal. At 1 hour, the arteriographic changes are so marked that they do not seem to fit the pattern of progressive vascular obliteration seen with all the other control burn groups. It is not until 6 or more hours post burn that one again encounters the devascularization seen in this group 1 hour post burn. Two hours post burn, arteriography demonstrates only slight differences from the immediate post burn group. These changes include some beading, discontinuity, and patchy loss of the finest branches of the arterial plexus. Tortuosity as noted by others is not present (Figure 5). At 4 and 6 hours (the difference is very slight but appears progressive) the arteriographic picture shows significant devascularization with only a few patent vessels left entering the region of the

burn. Communication across the burn is no longer shown. The central region is devoid of any patent vessels (Figure 6). At 24 hours post burn, the larger perforating arteries or short portions of their first branches can be seen coming to an abrupt end at the margin of the wound (Figure 8A). The wound itself is completely devascularized. This picture does not change at 48 or 72 hours.

2. Heparin-I. Arteriographs of the burn wounds of these animals treated with a single subcutaneous dose of 500 units of heparin immediately post burn cannot be distinguished from those of burned control animals at any of the times post burn that were studied.

3. Heparin-II. In this group of animals, pretreated for 24 hours and maintained post burn on heparin, the arteriographs departed from the pattern set by the burn control group. At time zero, this group appeared essentially like the controls both burned and unburned as did all immediate post burn groups. At 6 hours post burn, these specimens still showed many patent vessels penetrating the wound. These specimens more closely resembled the 2 hour post burn controls than the 6 hour post burn controls in their degree of devitalization (Figure 7). At 24 hours post burn, many vessels were still patent throughout the wound. This picture was not comparable to the 24 hour control which was completely devitalized. It resembled more closely the 4-6 hour post burn controls (Figure 8B). Between 24 and 48 hours, hemorrhagic patches appeared on the rats' ears and at the wound margins. Arteriographically, at 48 hours, the wound continued to resemble the 6 hour controls. At 72 hours, the circulation in the wound was obliterated as in the 24 to 72 hour post burn controls.

4. Heparin-III. Arteriographs of the burn wounds of these animals, maintained post burn with heparin but not pretreated, cannot be distinguished

from those of burned control animals at any of the times post burn that were studied.

5. Phentolamine. Three of the 20 rats burned and then given phentolamine died between the first injection (immediately post burn) and 6 hours post burn. In those that lived to 24 or 48 hours, patches on the ears became gangrenous. Six hours post burn, the arteriographs of these rats were indistinguishable from the 6 hour control animals. However, arteriographs of the rats sacrificed 24 hours post burn were more vascular than either the 24 or 6 hour post burn control (Figure 9). Patent vessels were diminished compared with unburned controls but they did not show the same kind of discontinuity as the 6 hour control. The 48 hour specimens were devitalized similar to the 24 and 48 hour controls.

6. Dextran-40. In rats treated with dextran immediately post burn, the group sacrificed immediately did not differ from the burned and unburned controls. At 6 hours post burn, the vasculature in the center of the burn appeared somewhat decreased compared with the 6 hour controls (Figure 10). At 24 and 48 hours, the wound was devitalized and comparable to the 24-72 hour controls.

7. Chlorpromazine. Arteriographs of the burn wounds of the animals treated with chlorpromazine post burn were indistinguishable from those of burned control animals at all of the times post burn that were studied.

8. Epinephrine. Arteriographs of the burn wounds of the animals treated with epinephrine post burn were indistinguishable from those of burned control animals at all of the times post burn that were studied.

DISCUSSION

Order and Moncrief[43] showed "immediate post burn" pictures which look more like the 4-6 hour post burn specimens in the present study. Since they were primarily looking at late changes in the burn wound vasculature associated with healing, it is possible that their immediate post burn animals were actually sacrificed as late as 6 hours post burn. It is also possible that their "immediate" specimens were done 1 hour post burn and that the 1 hour results in the present study represent a real, temporary, obliteration of vessels in the burn wound. Their 24-48 hour specimens appeared comparable to those reported here. These data suggest that the damage is essentially complete by 24 hours post burn and that a majority of the damage is done in the first 6 hours. These results agree with Jackson's observations[27] that the zone of stasis has become cut off from the circulation by 24 hours. They also agree with Parsons, Alrich and Lehman[45] and Goulian[18] who suggest that the zone of stasis is devitalized in the first 4-6 hours post burn.

The venous circulation, not studied here, may be important and deserves further study. The work of Massiha and Monafo,[37] published after the bulk of this research was done, confirms the fact that dermal vasculature is patent immediately post burn but indicates that progressive stasis begins immediately after the burn on the venous side. In agreement with their work, the disappearance of contrast from arterial circulation over time progressed upstream from the smallest vessels at the center of the burn to the larger ones at the periphery.

Heparin

In attempting to modify the stasis and occlusion of vessels in the burn wound, heparin is logically one of the first agents to try. With our present understanding of the pathophysiology of the burn wound, heparin should contribute in a number of different ways. First, and most obviously, it should prevent thrombosis secondary to the "sludging" found in the vessels of the burn wound. Preventing thrombus formation might prevent tissue anoxia and edema formation secondary to obstructed efflux of blood. It might have the effect of desludging the lymphatic drainage also. Secondly, heparin would help to maintain or reduce blood viscosity leading to improved circulation through what we must assume to be a compromised vascular bed. Thirdly, heparin is known to have an antihistaminic effect and a platelet sparing effect which prevents the release of 5-hydroxytryptamine from damaged platelets. These effects can counteract the inflammatory process affecting the vascular walls which lead to edema, hemoconcentration, and increased vascular resistance. Fourthly, heparin is known to act as a detoxifying agent for a number of substances including digitalis, curare, bee venom, cobra venom, congo red, and others. There is some evidence that many of the vascular phenomena and certainly the systemic effects associated with large burns may be mediated by a "burn toxin," perhaps a protein altered by the heat.

Heparin is a mucopolysaccharide molecule consisting of equal amounts of D-glucosamine and D-glucuronic acid, apparently in alternating configuration. It has a molecular weight of 6-20,000 and contains up to 40% esterified sulfuric acid. Variability of experimental results obtained using heparin may well reflect variability of molecular weight and/or percentage of esterified acid. Coating of cells and endothelium by this

negatively charged molecule increases mutual repulsion which may decrease viscosity and vascular resistance. Heparin occurs naturally in mast cells and may be released with histamine as a means of controlling its effects. In order of decreasing effectiveness, heparin inhibits: factor XI, factor IX, thromboplastin generation (probably a combination of its effects on factors V, IX and XI), factor V, thromboplastin, thrombin, and finally the aggregation of platelets by thrombin. Guest and Bond[21] demonstrated that vascular flow changes as well as changes in clotting and fibrinolysis in a burn could be duplicated by infusion of thromboplastin. This would indicate that the major effect of heparin in burns might be to counteract the release of tissue thromboplastin from damaged tissue.

McCleery[38] demonstrated that given 2 hours post burn heparin was of benefit in reducing the duration of vascular compromise by sludging from 4-5 days to 36 hours. Elrod, McCleery and Ball[16] demonstrated increased survival in animals receiving lethal burns. It also reduced hemoconcentration, increased lymphatic flow, and improved renal function. The survival times of animals given heparin immediately before the burn did not vary significantly from those of animals heparinized 2 hours post burn. Green and Artz[19] found that eschars sloughed and healing was completed sooner in rats given heparin daily beginning four hours post burn than in untreated animals. Conventional doses were also given to seven patients but results were difficult to evaluate without controls. Berberian[5] reported on a series of 40 patients with burns over 5-20% of their bodies. Saliba and Griner[49] reported that heparin appeared to reduce size or prevent extension of the burn wound as compared with the burn controls. Severity of tissue destruction and delay of healing was greater in the saline treated and control animals as compared with those treated with heparin. Later,

Saliba, Dempsey and Kruggel[50] reported clinical trials in seven patients with deep dermal and full thickness burns over 15-60% of the body. Fluid requirements were said to be significantly reduced and healing was reported to take less time. Obviously, there were no controls.

The fact that heparinization was only effective in delaying the onset of stasis when begun before the burn seems to confirm Massiha and Monafo's[37] finding that changes occur immediately post burn or intra-burn such that heparin given immediately post burn is too late to alter the stasis which begins on the venous side and affects the arterial circulation later as it progresses upstream. The fact that heparin pretreatment appears, arteriographically at least, to protect the circulation within the burn for as long as 48 hours, indicates that vascular thrombosis is a major component of circulatory stasis in burns. The failure of heparin to maintain patent wound vasculature at 72 hours indicates that thrombosis is not the only mechanism of stasis and that whatever else is involved is still exerting its effect as late as 72 hours post burn.

Phentolamine

In the search for agents to reverse vascular occlusion in a burn, it seems reasonable to attempt to dilate those vessels to allow sludged blood to pass and to preserve circulation in face of a lumen narrowed by layering of leukocytes and swelling of endothelial cells. As mentioned earlier, flow varies as the fourth power of the radius of the vessel. Jackson,[27] and Myers and Cherry[42] showed that getting oxygen to the injured tissue does not seem to be enough to ensure survival. Maintaining flow to the area seems to be a critical factor. Muscular arterioles and the "precapillary sphincters" seem to constrict primarily in response to alpha-adrenergic stimulation. The role of cholinergic fibers identified in association

with small vessels is not clear. It is reasonable to postulate increased catecholamine circulation following a burn. It is also reasonable to assume that neurons affecting cutaneous circulation are damaged in the full thickness burn. In that case, circulating catecholamines have the potential to act unopposed in denervated vessels. For this reason it was felt that alpha-adrenergic blocking agents should be capable of improving the circulatory picture within the burn wound. It was hoped that the use of an alpha blocker would return the vessels to a normal or supra normal dilation allowing for increased flow to the area.

Phentolamine is a short acting alpha-adrenergic blocking agent. It is capable of increasing peripheral blood flow particularly in the skin. Some of the vasodilator effect may be due to a direct action on the vascular smooth muscle. A prominent side effect of this drug is cardiac stimulation. It does not appear to inhibit adrenergic stimulation of the heart.

Palmer,[44] studying oversize skin flaps in rats, attempted to increase endogenous catecholamine release by subjecting his rats to periods of stress. Under these circumstances, flap survival was significantly better in rats given phentolamine just before and after each period of stress than in control animals stressed but untreated. He describes two types of denervation hypersensitivity to adrenergic stimulation. Late hypersensitivity occurs 7-14 days after the denervation, is non-specific, and is mild in degree. Early hypersensitivity develops within the first 24-48 hours and is a result of the inability of damaged neurons to take up surplus amines. This impaired uptake must increase the concentration of catecholamine available to affect the vessels. Myers and Cherry[42] demonstrated increased flap survival using phenoxybenzamine, a longer-acting alpha-blocker, without the use of additional stress. Green and Ginsburg[20]

demonstrated that inflammatory effects in thermal injury, particularly edema formation, seemed to be mediated by adrenaline. This effect could be opposed effectively by pre-treatment with phenoxybenzamine. Robb[48] demonstrated that many of the changes he observed post burn could be reversed both by heparin and phenoxybenzamine.

The effect of phentolamine cannot easily be explained. From the failure of chlorpromazine to alter vascular changes in the burn wound, one may assume that the alpha blocking effects of phentolamine are not its main method of action in preserving the arterial vasculature at 24 hours post burn. It is possible that the alpha blocking effects may have been the cause, either directly or by systemic reflex, of the demise of the wound vasculature between 24 and 48 hours. The deaths of three rats after their first injection, and the necrotic patches on the ears of the 48 hour rats may have been due to systemic hypotension and hypoperfusion respectively in response to the alpha blocking effect of phentolamine. The effect of phentolamine not duplicated in chlorpromazine is its cardiac stimulating effect. It is possible that this is the reason for sustained patency of wound vessels with phentolamine. The collapse of these vessels after 24 hours may be due to continuing fluid loss via the burn for which the cardiac stimulating effect could no longer compensate.

Dextran-40

The dextrans were introduced in the 1950's as a potential plasma expanding agent. Since that time, studies have revealed that different molecular weight fractions have slightly different properties.[6,11] Dextran fractions currently in favor for clinical use are the 40,000 and 70,000 molecular weight units. Dextran 40 is of interest in this work for three important actions. First, and most important, the dextrans act to

preserve flow through the microcirculation in shock conditions. Second, they act as plasma expanders. Finally, they seem to have an antithrombotic (anti-sludging) effect on the blood.

In preserving microcirculatory flow, dextran 40 is much more effective than dextran 70. Part of its effect is to increase perfusion pressure by expansion of plasma volume. The majority of the effect, however, is in reducing viscosity of the blood.[4] The more viscous the blood, the more dramatically the flow drops with progressive decrements in perfusion pressure. Because blood is a non-Newtonian fluid, this effect is exaggerated in the smaller vessels. Dextran 40 is actually more viscous than normal plasma. However, this viscosity is modified by its hemodiluting effect. In addition, dextran 40 reduces viscosity by prevention of aggregations of formed elements of the blood by coating them and the endothelium, decreasing the attraction between them. Dextran 40 also has an effect of increasing the malleability of the erythrocytes which allows them to squeeze more easily through narrowed or deformed capillaries.

As a plasma expander, dextran 40 is inferior to dextran 70 because the smaller molecular size allows some to pass across membranes and to be excreted relatively rapidly by the kidney. Dextran 40 actually has more particles per unit weight than dextran 70 but this is not sufficient to overcome the effect of transmembrane losses. Nonetheless, the effect is present for up to three hours. Plasma expansion is important both for inhibiting "sludging" by increasing the space between formed elements, and for increasing total volume available to the cardiac pump.

Several mechanisms have been postulated for the antithrombogenic effects of dextran 40. First, the reduction of viscosity leads to an increased flow rate. This prevents particles from aggregating by decreasing

the time during which attractive forces are at work. Second, it coats the cells and the endothelium preventing the action of attractive forces between them. Third, hemodilution is effective in separating particles both reducing forces between them and increasing flow. Finally, some observers have felt dextran 40 leads to a reduction of extra fibrinogen and other clotting factors by an undetermined mechanism.[4]

Dextran 40 seemed to have merit before it was tried. It was very surprising to see that, if anything, it was detrimental to the preservation of the microcirculation of the burn. Because it is a small enough molecule to cross membranes, it is possible that under the conditions of increased permeability of vessels in the wound, enough dextran was lost into the tissue to oppose its volume expanding effect and perhaps even to contribute to local edema.

Chlorpromazine

The use of chlorpromazine in our work was prompted by the work of Horn and Converse.[24] Chlorpromazine is best known for its anti-psychotic and sedative effects but it is also a potent vasodilator and a weak antihistamine among other things. The vasodilating effects are very likely due to an adrenergic blocking effect. Chlorpromazine produces a characteristic competitive alpha-adrenergic blockade *in vitro*. The effect is less reliable *in vivo* due to the many other interacting factors. There is also possible a direct effect of chlorpromazine on the vascular smooth muscle. Chlorpromazine also has local anesthetic properties.

Horn and Converse used chlorpromazine for its vasodilating effect in an attempt to alter survival in lethal burns in rats. Their results showed a dramatic reduction of the acute (less than 24 hours) deaths but a greater cumulative mortality at 48 hours and beyond in chlorpromazine treated rats

as compared with their fluid treated controls. Chlorpromazine seemed to protect against early death exhibited by the untreated controls better than fluid therapy but appeared to be deleterious to long term survival. The authors suggest that perhaps better results would be achieved with a much reduced dosage on the second day.

This work is not directly relevant to the present study because the authors were dealing with systemic effects of lethal burns and the ability of chlorpromazine to modify them. In the present work, the intent was to modify local changes in a more moderate burn. The attempt here was not to decrease mortality but to decrease morbidity. Nevertheless, the same pharmacologic effects were sought at the microcirculatory level. Chlorpromazine might prevent vascular stasis by preventing or relieving vascular spasm. The antihistaminic effects may be an added bonus which might help to decrease the endothelial leakage and edema formation which contribute to vascular stasis. Lokkegaard, _et al_,[35] demonstrated that pretreatment with chlorpromazine led to decreased vascular resistance and increased viability of kidneys following one hour of warm ischemia. This work was encouraging because it dealt with a reversal of ischemic changes in a local vascular bed. It was less valuable for the present work because it involved pre-treatment with the chlorpromazine while the present work was a search for an agent to be given post burn.

Epinephrine

The use of epinephrine in the prevention or reversal of microcirculatory stasis post burn seems, at first glance, to be contraindicated. Epinephrine is well known as a vasoconstrictor. Yet the task at hand was to prevent or reverse vasospasm and occlusion. However, Reinisch[47] has shown that very dilute epinephrine in concentrations between $1{:}4\text{x}10^6$ and $1{:}10^7$ selectively

constricted patent arteriovenous anastomoses (AVA's) in oversized skin flaps of pigs. Constriction of AVA's diverts flow into the nutrient capillary beds. When the AVA's are fully dilated, tissue may become ischemic despite measurable blood flow because nutrient exchange does not take place across these thicker walled vessels.

Reinisch's work with skin flaps points out that the response to sympathectomy in skin flap tissue is immediate dilation of the AVA's. This gives the impression that there is increased flow through the tissue and, in fact, there is. However, this increased flow is non-nutritive and the tissue dies. The full thickness burn wound may be assumed to be a sympathectomy of the dermal vessels. It is therefore possible that the presence of flow with the decreased reduction of oxyhemoglobin observed by Jackson[27] in the zone of stasis is due to the diversion of flow from the nutritive beds through the AVA's. Myers and Cherry[42] in an earlier article demonstrated that patent vessels by arteriography was no guarantee of skin flap survival.

The present work differs from Reinisch's work in several important respects. Reinisch studied the use of epinephrine in skin flaps which is similar to the burn only in the presumed denervation of the sympathetics. While vascular change is a response to flap formation, uniform vascular injury is not a major component of the skin flap. In the burn, vascular change is part of the injury. Also, Reinisch injected epinephrine locally in his skin flaps. Systemic effects were avoided in this manner. This study infused the epinephrine intravenously for practical reasons (repeated local injections would have required repeated anesthetics or the stress of restraint). This method raises questions about systemic reflex involvement in the circulatory changes of the burn. It is also possible that none of

the epinephrine ever arrived at the appropriate receptor site either because of systemic mono amine oxidases or because of the anatomical site of the receptors.

Technique

The technique used in this study has several limitations. The subjective nature of the data is not a drawback for the present work but it may be less useful for future investigation if the degree of injury of the microcirculation is to be measured. There is no way to assign numbers to burn wound arteriographs and therefore no way to establish standard deviations. The present work was designed to establish whether changes occurred. This could be done without numerical analysis.

Arteriography as performed in this work is a static study. Circulation is dynamic. The technique used here tells very little about the intravascular events following a burn except whether dye is able to penetrate the small dermal vessels within the wound. Heparinized rats exhibit clotting times far in excess of normal, yet in the heparin III group, arteriographic dye was unable to penetrate the circulation of the wound. It is not possible from the arteriographic data to say whether it was clotting or some other process that caused the isolation of the wound circulation. Furthermore, Reinisch[47] demonstrated that patent vessels on arteriography in skin flaps were no guarantee of tissue survival. Supravital microscopy would be a better, although no less subjective method for studying dynamic changes in the microcirculation of burns. The two techniques are complementary. Arteriography may give an overview of the extent of damage with microscopy demonstrating intravascular manifestations of that damage.

The burn model used here has been used in the past to produce a 20% full thickness wound. In rats, this is a non-lethal burn severe enough to

be at the verge of causing significant side effects, particularly hemodynamic ones, remote from the wound itself. Systemic effects presumably feed back on the wound which caused them. The addition of these systemic variables to the pathophysiology of the burn wound might be avoided by using a smaller burn.

Another modification of the burn model that might be helpful for this type of study would be to reduce the severity of the burn. Ideally, this burn would be severe enough for the zone of stasis to extend below the deepest epidermal elements with the zone of coagulation sparing these deep epidermal elements. Failure to salvage the zone of stasis with any experimental technique would result in full thickness injury while any success would result in partial thickness injury. The burn used in this work is probably extreme although there is no way of knowing how deep the division between the zone of stasis and the zone of coagulation goes since both slough. Somewhere between 10 seconds at 75°C, known to give partial thickness wounds, and the 15 seconds at 100°C used here, it would make sense to pick a burn just sufficient to produce a full thickness injury consistently.

It is clear, from the literature review, that more attention must be paid to the contribution of the venous circulation to burn stasis. Venous circulation has received less attention in medical research than the arterial side. Arterial circulation is generally felt to be the more dynamic with the venous system merely a passive network by which blood returns to the heart. It is probably as active in controlling the circulation as the arterial circulation but in a different way. Lymphatic drainage has received even less attention than the venous system but perhaps its activity is significant in controlling arterial and venous dynamics.

The work of Brauer and Spira[9] and Jackson[28] in getting zone of stasis tissue to support a graft suggests that the ultimate salvage of this tissue may involve physical as well as pharmacological manipulation of the tissue. It is fascinating to speculate about the mechanisms by which the graft influences the recovery of the static circulation. Zawacki[56] believes it is all a question of local fluid loss. It is probably not that simple. He is probably correct implying that local fluid balance is not reflected systemically. This concept needs to be explored.

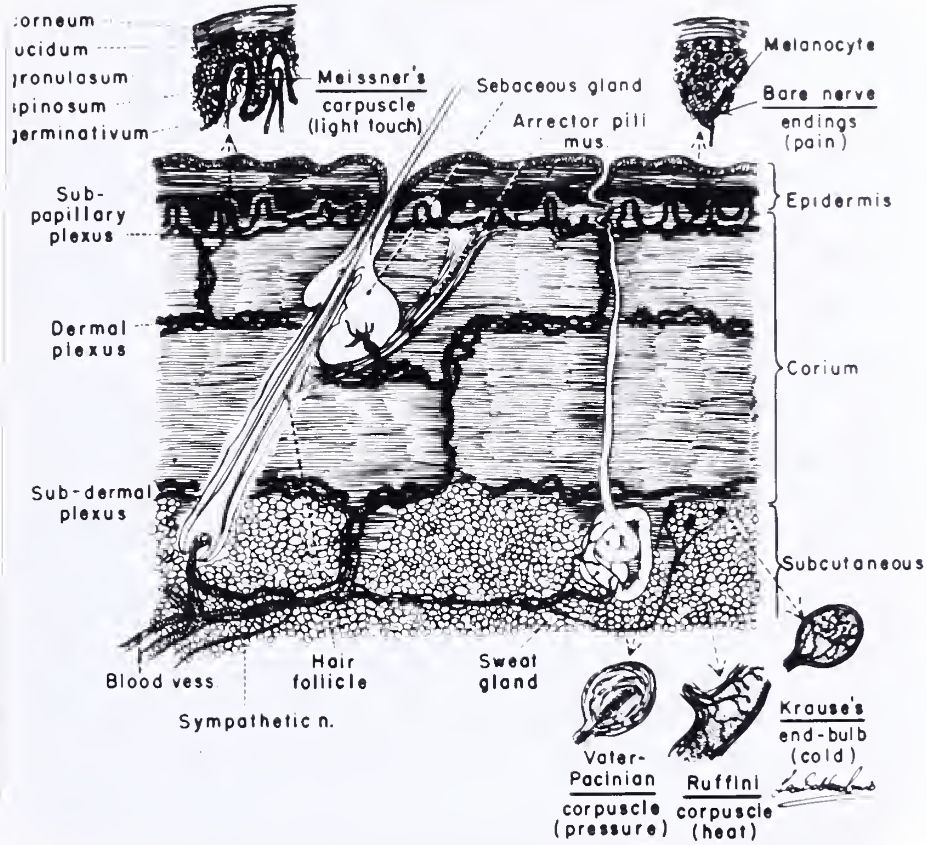
The most exciting aspect of this study is that it demonstrated that the burn wound can be manipulated pharmacologically. The experience of the heparin study indicates that if an agent can be found to prevent the progression of stasis, this agent must be present in the burn very early, at least sooner than heparin given subcutaneously immediately post burn. It is very encouraging that the intramuscular injection of phentolamine given immediately after the burn seems to have had some effect. This holds out hope that viable zone of stasis tissue demonstrated to exist by Brauer and Spira[9] may be salvageable. The list of possible agents which might be tried as well as combinations of agents, none of which were tried in this study, is lengthy. Until a "cocktail" is found which, given immediately post burn, reverses zone of stasis changes, it will not be possible to investigate the timing or critical period after which no therapy will be effective. Based on the results of this study, I would speculate that the critical period might fall around four hours. Massiha and Monafo's[37] results would suggest it might be earlier.

SUMMARY

Rats receiving a full thickness burn were studied arteriographically post-mortem first to repeat the work of Order and Moncrief[43] and secondly to see whether circulatory isolation of the burn wound observed by them could be altered by pharmacologic agents. Animals were sacrificed immediately and at 1, 2, 4, 6, 24, 48 and 72 hours post burn. Vascular compromise was first evident between 2 and 4 hours, and was complete by 24 hours. Chlorpromazine and dilute epinephrine failed to modify arteriographic appearance of the wound in any way. Dextran 40 appeared to decrease the vascularity of the burns 6 hours post injury as compared with controls. Phentolamine appeared to spare vessels from further damage between 6 and 48 hours. Heparinization was able to spare the vessels until 72 hours post burn but only if initially given before the burn.

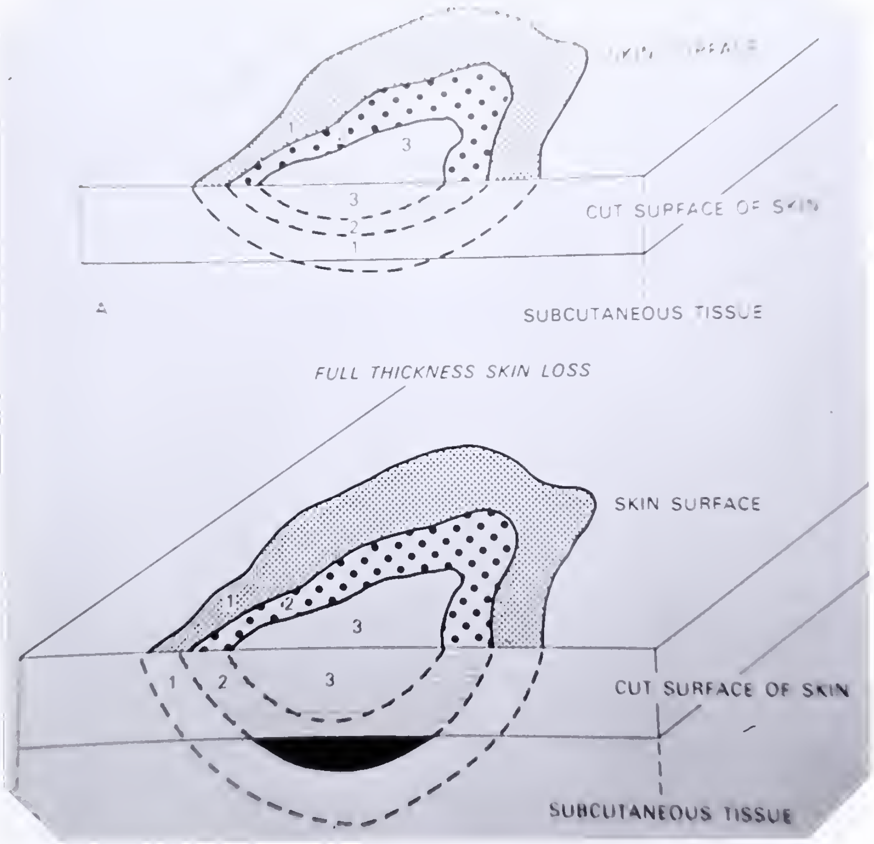
A review of the literature suggests that a study of the venous side of the circulation would be helpful in understanding the circulatory events within the burn wound and in selecting agents for future trials. It also suggests that physical as well as pharmacologic manipulation may be required to salvage zone of stasis tissue. Finally, it is felt that the present results, while not entirely successful at preventing burn wound stasis, indicate hope that the burn wound microvasculature can be pharmacologically manipulated to the benefit of the patient.

Figure 1



Normal Skin Anatomy (from Krizek, Robson and Wray, in Management of Trauma, ed., W. F. Ballinger, W. B. Saunders Company)

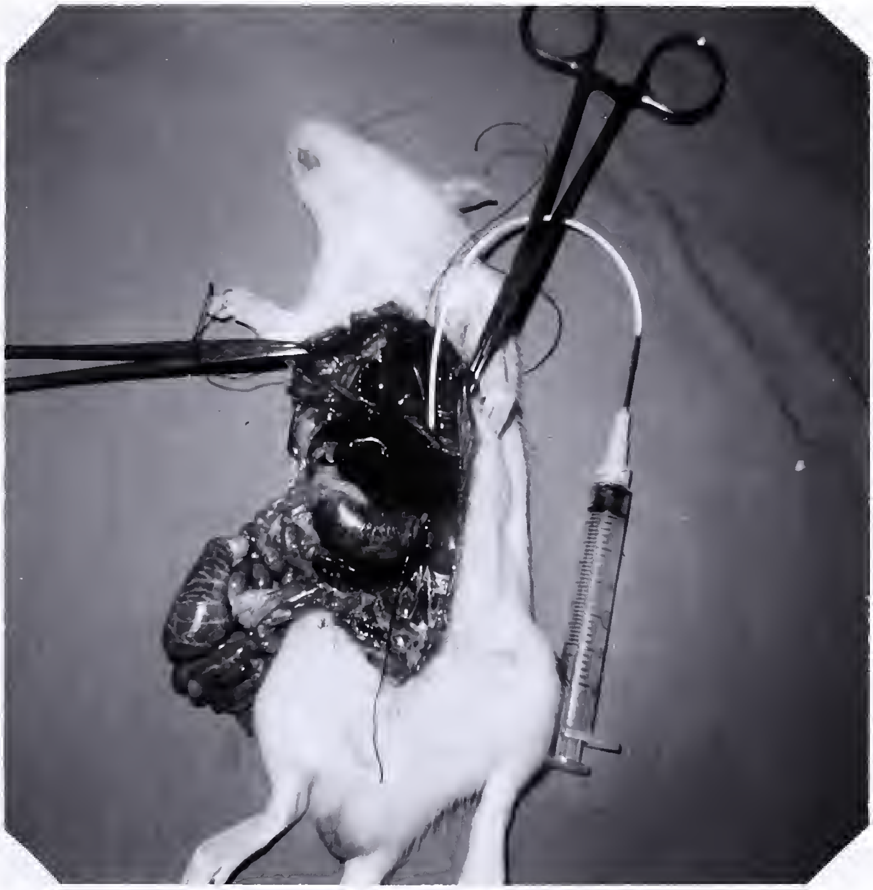
Figure 2



Jackson's Three Zones[29]

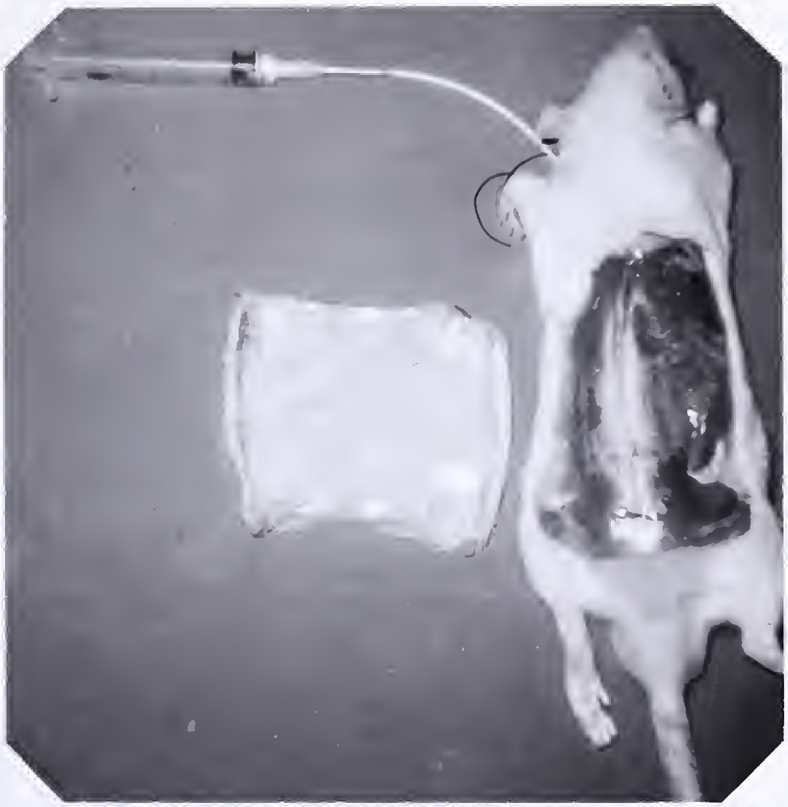
1. Zone of Hyperemia
2. Zone of Stasis
3. Zone of Coagulation

Figure 3A



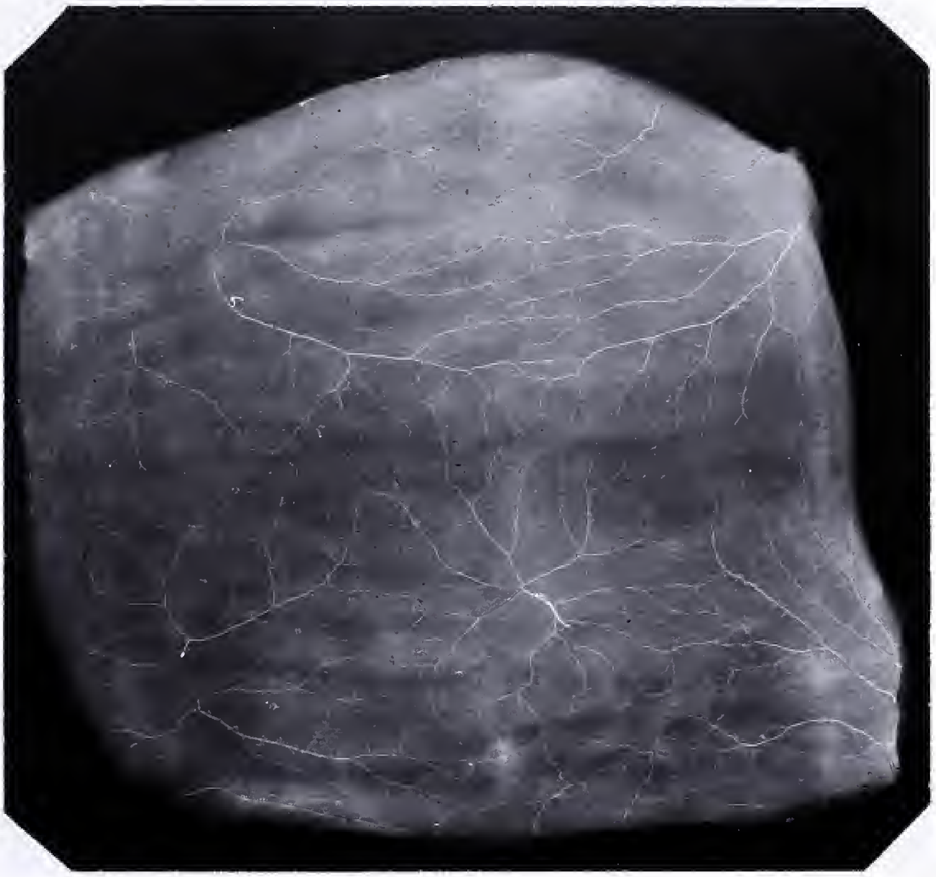
Injection of Micropaque:
Rat with Cannula in Descending Aorta

Figure 3B



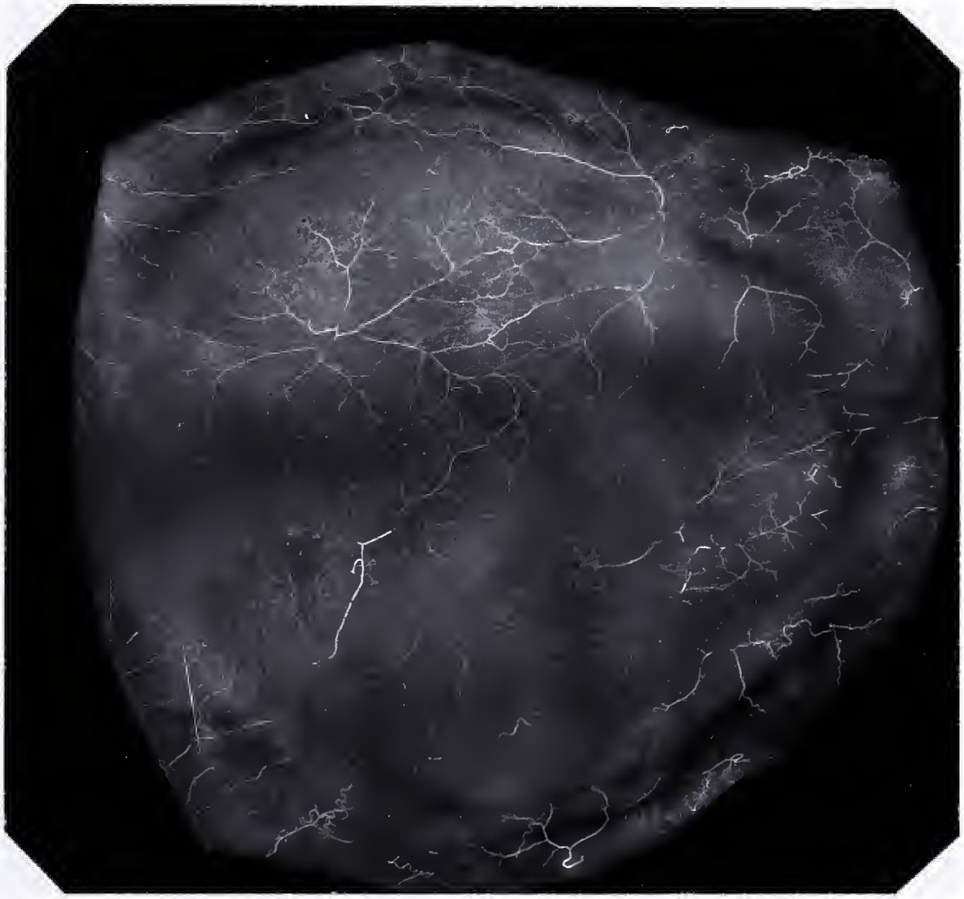
Rat with Burn Wound Excised at
the Depth of the Panniculous Carnosus

Figure 4



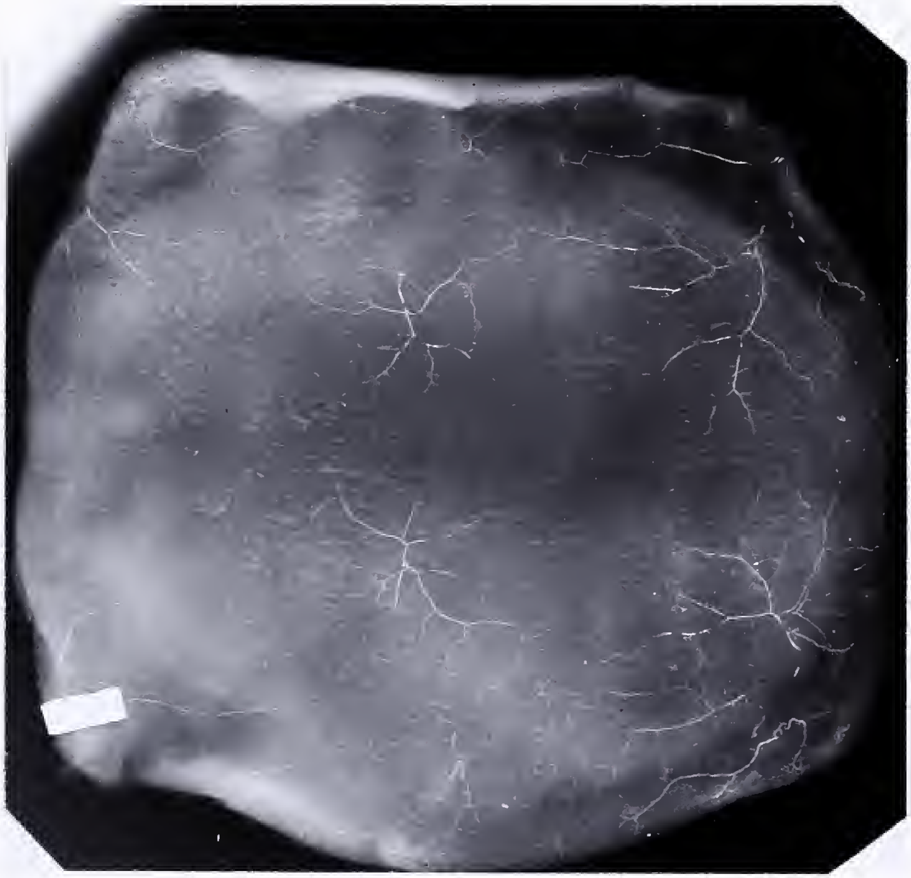
Unburned Control

Figure 5



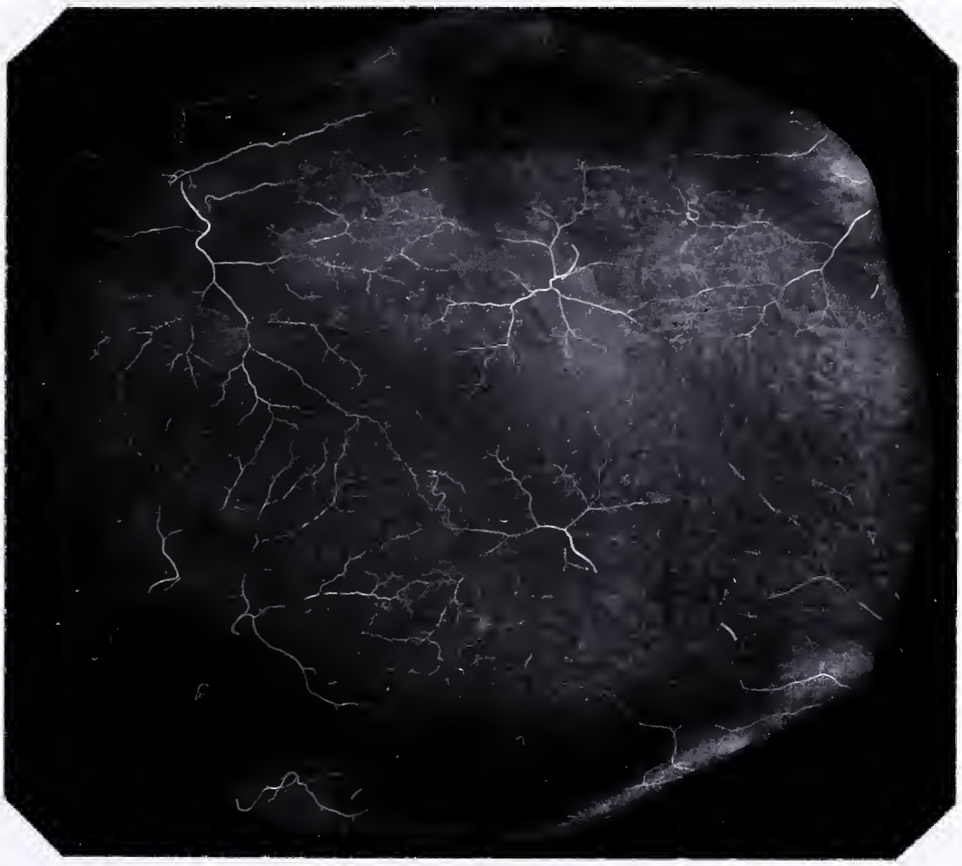
2 Hours Post Burn: Untreated Control

Figure 6



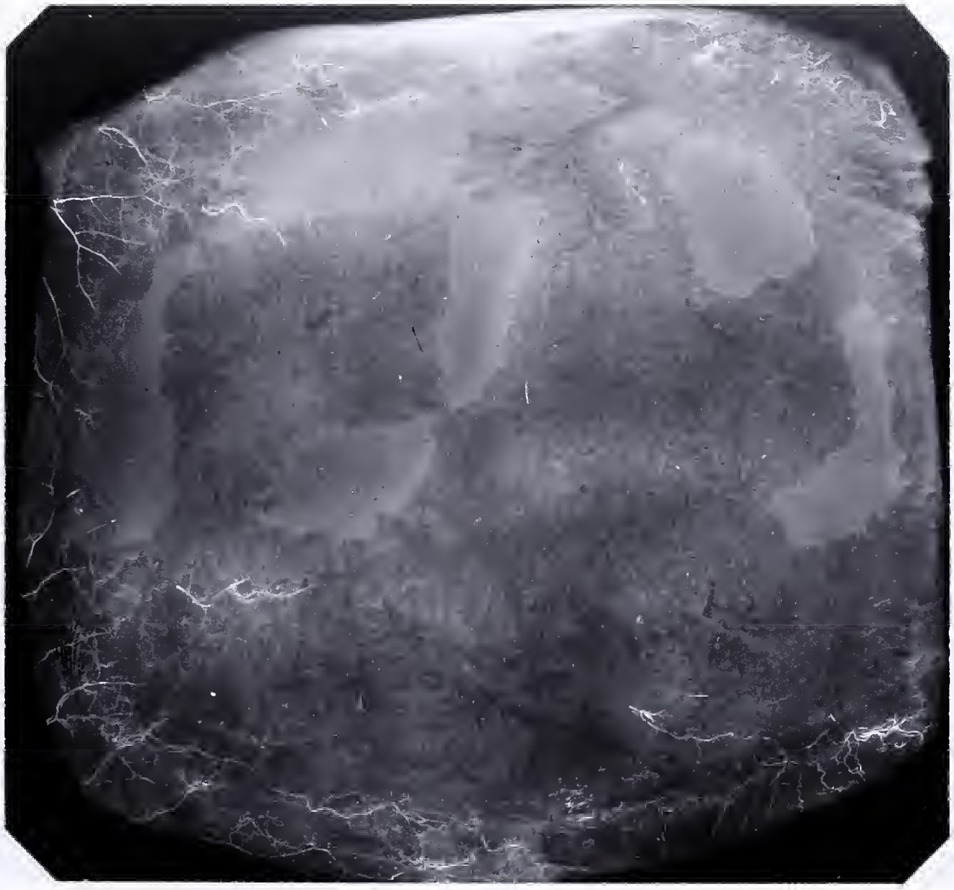
6 Hours Post Burn: Untreated Control

Figure 7



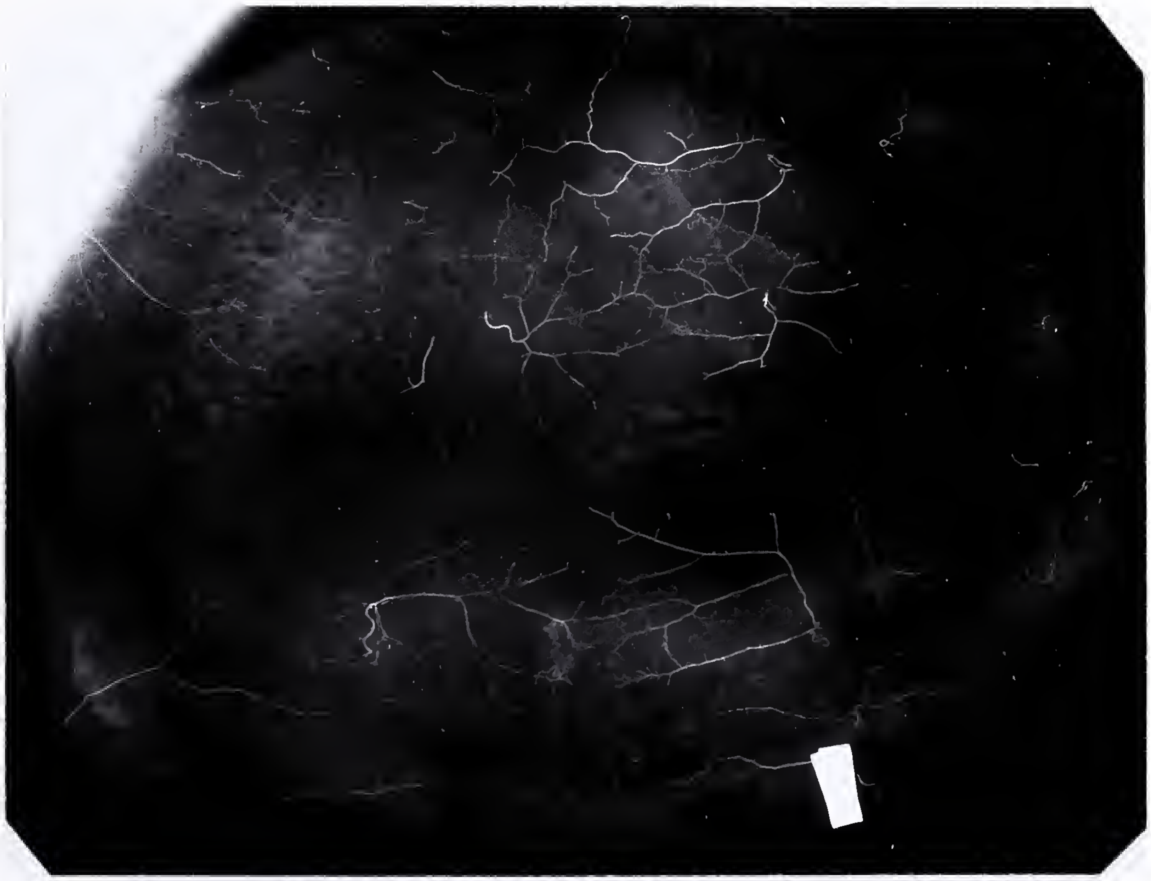
6 Hours Post Burn: Pretreated for 24 Hours and Maintained Post Burn with Heparin

Figure 8A



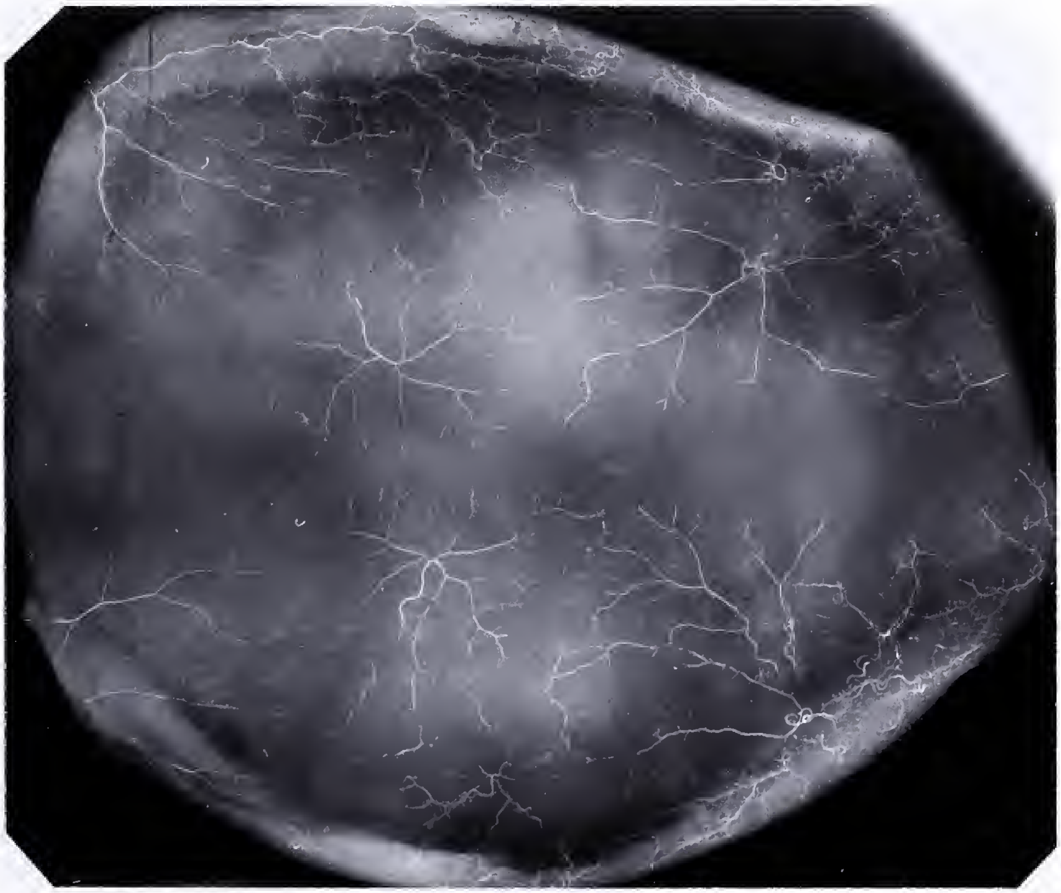
24 Hours Post Burn: Untreated Control

Figure 8B



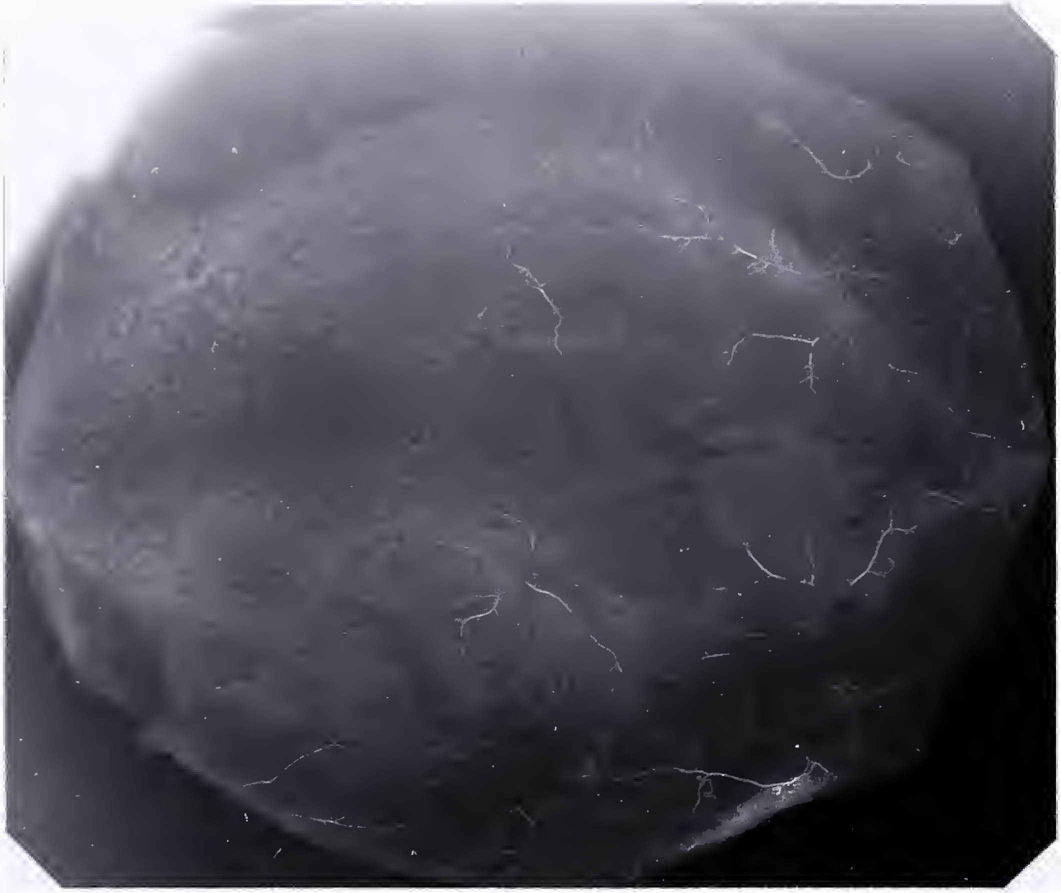
24 Hours Post Burn: Pretreated for 24 Hours and Maintained Post Burn with Heparin

Figure 9



24 Hours Post Burn: Phentolamine

Figure 10



6 Hours Post Burn: Dextran 40

APPENDIX

Micropaque Gelatin

- Micropaque Gelatin
  - 10 gm. mucilage acacial
    - 4 gm. gum acacial
    - 40 gm. syrup
      - 850 gm. sugar, add H_2O to make 1000 cc. solution
  - 15 gm. gelatine powder
  - 200 gm. water
  - 150 gm. micropaque

In making the mucilage acacial the syrup may have to be heated to dissolve the sugar with the water. The solution should not be allowed to settle when adding gum to the syrup. After the other materials are added the entire mixture should be placed in a blender for approximately twenty minutes.

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